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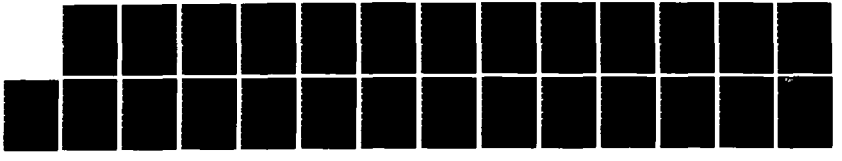

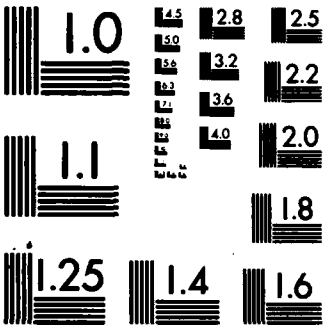

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One-hundred ml aliquots of baboon whole blood collected in 14 percent citrate-phosphate-dextrose (CPD) anticoagulant solution in plastic bags were stored at 4 C for 28 days in the presence or absence of 0.75 grams of phosphate anion exchange resin. In vitro measurements and in vivo measurements after auto-transfusions were made to determine whether the phosphate anion exchange resin had any beneficial effect on the blood.
The in vitro measurements of red cell 2,3 DPG and P50 were higher throughout the 28 days of storage at 4 C in the blood stored in the phosphate anion

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exchange resin. Twenty-four-hour posttransfusion survival measurements in six baboons after autologous transfusions of red blood cells prepared from whole blood stored at 4 C for 21 days showed a mean of 86 ± 6 percent in the presence of resin and 83 ± 6 percent in the absence of resin. In five other baboons, red blood cells prepared from 28-day-old blood showed a mean 24-hour post-transfusion survival value of 82 ± 4 percent in the presence of resin and 75 ± 4 percent in the absence of resin.

The addition of a phosphate anion exchange resin to the CPD anticoagulant provided better maintenance of red cell 2,3 DPG and P_{50} levels during storage of whole blood at 4 C, and red blood cells prepared from whole blood stored in this solution had better oxygen transport function than red blood cells prepared from blood without resin. Red blood cell ATP levels and 24-hour post-transfusion survival values were similar whether or not the anticoagulant contained resin.

ABSTRACT

One-hundred ml aliquots of baboon whole blood collected in 14 percent citrate-phosphate-dextrose (CPD) anticoagulant solution in plastic bags were stored at 4 C for 28 days in the presence or absence of 0.75 grams of phosphate anion exchange resin. In vitro measurements and in vivo measurements after autotransfusions were made to determine whether the phosphate anion exchange resin had any beneficial effect on the blood.

The in vitro measurements of red cell 2,3 DPG and P_{50} were higher throughout the 28 days of storage at 4 C in the blood stored in the phosphate anion exchange resin. Twenty-four-hour posttransfusion survival measurements in six baboons after autologous transfusions of red blood cells prepared from whole blood stored at 4 C for 21 days showed a mean of 86 $\pm$ 6 percent in the presence of resin and 83 $\pm$ 6 percent in the absence of resin. In five other baboons, red blood cells prepared from 28-day-old blood showed a mean 24-hour posttransfusion survival value of 82 $\pm$ 4 percent in the presence of resin and 75 $\pm$ 4 percent in the absence of resin.

The addition of a phosphate anion exchange resin to the CPD anticoagulant provided better maintenance of red cell 2,3 DPG and P_{50} levels during storage of whole blood at 4 C, and red blood cells prepared from whole blood stored in this solution had better oxygen transport function than red blood cells prepared from blood stored without resin. Red blood cell ATP levels and 24-hour posttransfusion survival values were similar whether or not the anticoagulant contained resin.

INTRODUCTION

Glucose and adenine have been added to the CPD preservative in an attempt to extend the shelf-life of red blood cells at 4 C. The CPD anticoagulant without the supplement maintains red cell viability for 21 days; the supplemented CPD solution maintains red blood cell viability at 4 C for 35 days.[1] Supplementation of the anticoagulant does not protect the functional property of the red blood cells during 4 C storage. Anticoagulants are not intended for maintenance of 2,3 DPG or blood pH, and there is a significant reduction in these levels after about 10 days of storage at 4 C.[2-4] Anticoagulants are employed primarily to maintain the ATP level of the red blood cells and to ensure 24-hour posttransfusion survivals of at least 70%.

It has been reported that human blood stored at 4 C in the CPD anticoagulant to which phosphate anion-exchange resin had been added showed satisfactory maintenance of blood pH and red blood cell 2,3 DPG for two weeks; red blood cell viability was not reported.[5]

Red blood cell function can be evaluated from in vitro measurements of 2,3 DPG and P_{50} values, but the posttransfusion survival of the preserved red blood cells cannot be determined either from in vitro biochemical measurements of ATP or 2,3 DPG or from physical-structural measurements of osmotic fragility, mechanical fragility or morphology.[6-10] In vivo studies must be done to measure posttransfusion survival values, and healthy human volunteers generally have been used in these in vivo studies. Now, however, some investigators are using the baboon as an

initial test model before performing in vivo studies in humans, because of observed similarities between human and baboon red blood cells.[11-19]

In the study reported here, we made in vitro measurements and measured in vivo circulation of autologous baboon red blood cells prepared from whole blood that had been stored at 4 C for 21 to 28 days in the presence and absence of the phosphorylated ion-exchange resin.

MATERIALS AND METHODS

Eleven healthy male baboons of the species Papio cynocephalus, weighing from 20-30 kg, were used in the study. Prior to phlebotomy each baboon was sedated with 30 mg of phencyclidine hydrochloride (Sernylan) or with 130 mg of pentobarbital. Using an 18-gauge needle and an AE-2 connector set, a 100 ml volume of peripheral venous blood was drawn into a sterile 150 ml polyvinylchloride (PVC) plastic transfer bag containing 14 ml of CPD anticoagulant, and the whole blood was stored at 4 C for 21 to 28 days in the PVC plastic bag. Some of the units were stored in the CPD anticoagulant without resin, and others in CPD with 0.75 grams of phosphorylated ion exchange resin (Cutter Laboratories, Emeryville, CA) supplied in a dialysis membrane pouch approximately 1 X 2 X 3 cm in size. Samples were obtained from the unit of blood on the day of collection (day 0), and 7, 14, 21, and 28 days after collection, for measurements of red blood cell ATP, 2,3 DPG, P_{50}, blood pH, glucose and lactate, and plasma and blood inorganic phosphate levels. The blood was agitated before each sample was taken.

Nine baboons received autotransfusions of red blood cells prepared from blood stored in CPD with resin, and nine baboons received red blood cells prepared from blood stored without resin, and measurements were made of ^{51}Cr 24-hour posttransfusion survival and lifespan as described previously.[20,21] The length of time in days when 50% of the red cell-associated radioactivity disappeared from the blood is reported as the T_{50} value. Red blood cell ATP and 2,3 DPG levels were measured as

previously described.[22-24]

The red blood cell affinity for oxygen of washed red blood cells in phosphate-buffered saline, pH 7.2, was measured at a temperature of 37 C and a pCO_2 tension of 0 mm Hg using the Hemoscan Oxygen Dissociation Analyzer (American Instruments Co., Silver Spring, MD).[25] The pO_2 tension at which 50% of the hemoglobin was saturated with oxygen is reported as the P_{50} value. Blood pH, pCO_2, and pO_2 determinations were performed at 37 C on the IL 813 Blood Gas Analyzer (Instrumentation Laboratory, Inc., Lexington, MA). Perchloric acid filtrates were prepared from blood for glucose and lactate determinations. Glucose was measured using the glucose oxidase method. Lactate was determined as described by Hohorst using a spectrophotometric procedure.[26] Inorganic phosphorus levels in blood and plasma were determined spectrophotometrically.[27]

The in vitro data were analyzed by non-paired t-test, and the 24-hour posttransfusion survival and T_{50} values were analyzed by either the non-paired or the paired t-test.

RESULTS

Throughout the 28 days of 4 C storage, the units stored in CPD with resin showed consistently higher pH's than those stored in CPD without resin (Table 1 and Figure 1). The initial pH of the blood stored in the resin-supplemented anticoagulant was 7.1; on the 28th day of storage it was 6.9. The blood stored in CPD without resin had an initial pH of 7.0; the pH was 6.7 on the 28th day of storage. After 28 days of blood storage at 4 C, red blood cell ATP levels were similar whether or not resin was used in the anticoagulant (Table 1 and Figure 2).

TABLE 1
FIG. 1

FIG. 2

Measurements of red blood cell 2,3 DPG and P_{50} made after 14, 21 and 28 days of 4 C storage were significantly higher in the blood with resin, and in each group there was a parallel between the red blood cell P_{50} value and the red blood cell 2,3 DPG level (Table 1).

Glucose utilization was greater in the blood with resin: after 28 days of 4 C storage the blood with resin contained 141 mg percent of glucose compared with 176 mg percent in the blood without resin. The glucose levels in the blood with resin were consistently lower throughout the period of 4 C storage, although the differences were not statistically significant (Table 1).

Blood lactate levels during the 28 days of storage at 4 C were not statistically different between the two groups (Table 1), although interestingly the levels were somewhat higher in the blood with resin even though this blood had higher pH levels. On the 7th and 14th days of storage, the blood with resin exhibited statistically significant

reductions in pCO_2 levels (Table 1). Throughout the 28 days of 4 C storage, blood and plasma levels of inorganic phosphate were higher in the blood with resin (Table 1).

The mean 24-hour posttransfusion survival value was 86 ± 6 percent for autologous red blood cells prepared from blood stored with resin and 83 ± 6 percent for those prepared from blood stored without resin for 21 days (Table 2). When the storage period was 28 days, the mean 24-hour posttransfusion survival value was 82 ± 4 percent for autologous red blood cells prepared from resin stored blood and 75 ± 4 percent when resin was not utilized during blood storage (Table 2).

TABLE 2

DISCUSSION

Human red blood cells and baboon red blood cells preserved in a similar manner exhibit similar 24-hour posttransfusion survival values.[17] In the study reported here, the addition of resin to the CPD anticoagulant before storage of baboon blood at 4 C provided better maintenance of red blood cell 2,3 DPG and P_{50} throughout 28 days of storage. Red blood cell ATP levels and posttransfusion survival values were similar whether or not the anticoagulant was supplemented with resin.

The blood treated with resin had slightly higher blood lactate levels than the blood without resin. A previously reported observation that an accumulation of lactic acid causes a decline in pH during blood storage at 4 C[28] was not borne out in our study; we saw higher pH levels throughout the storage period in the blood treated with resin. The higher pH in the blood might have been due to a slow release of monohydrogen phosphate anion ($HPO_4^=$) from the resin during storage. Inorganic phosphate is known to stimulate glycolysis in red blood cells,[29,30] and this was reflected in the higher lactate level and greater glucose utilization in the blood stored in the resin for 28 days.

The higher phosphate levels observed in the whole blood and plasma in the presence of resin throughout the 28 days of storage reflected the release of inorganic phosphate. Although inorganic phosphate usually has a deleterious effect on 2,3 DPG and a beneficial effect on ATP,[31-33] in this study the slow release of phosphate anions by the resin helped to maintain blood pH and reduce blood pCO_2 levels, and facilitated

maintenance of 2,3 DPG and P_{50}. An increase in the blood pCO_2 level in the blood without resin paralleled a decline in blood pH independent of lactic acid.

The red blood cells prepared from baboon blood after storage in the liquid state in the presence of phosphate ion exchange resins exhibited superior oxygen transport function, but the ATP levels and posttransfusion survival values were similar whether or not the anticoagulant was supplemented with resin.

The baboon can serve as an excellent model for in vivo studies of red blood cells subjected to modified anticoagulants, new plastic containers, and new freezing methods. The safety and effectiveness of these variables can be determined in the baboon before studying human volunteers.

TABLE 1

Red Cell 2,3 DPG, ATP, P_{50}, Blood pH at 37 C, Blood Glucose, Lactate,and Phosphate,and Plasma Phosphate, and pCO_2 Levels,in Baboon Blood Stored for 28 Days With and Without 0.75 g Resin

			Day 0	Day 7	Day 14	Day 21	Day 28
2,3 DPG (uM/g Hb)	Control	Mean	15.85	9.47	2.74	0.89	0.60
		SD	2.96	4.10	1.80	0.37	0.52
		SE	0.99	1.45	0.60	0.12	0.20
		n	9	8	9	9	7
	Resin	Mean	15.08	13.91	12.19	5.59	2.36
		SD	2.59	4.45	4.42	2.31	1.13
		SE	0.86	1.57	1.56	0.77	0.40
		n	9	8	8	9	8
		df	16	14	15	16	13
		t	0.59	2.08	5.91	6.03	3.79
		p	>0.50	>0.05	<0.002	<0.002	<0.01
ATP (uM/g Hb)	Control	Mean	4.22	3.96	3.58	3.26	2.46
		SD	0.65	0.98	0.88	0.87	1.02
		SE	0.21	0.33	0.29	0.29	0.39
		n	9	9	9	9	7
	Resin	Mean	3.81	3.56	2.86	2.94	2.55
		SD	0.78	1.40	0.86	0.99	1.35
		SE	0.26	0.53	0.29	0.33	0.48
		n	9	7	9	9	8
		df	16	14	16	16	13
		t	1.23	0.68	1.77	0.73	0.15
		p	>0.20	>0.50	>0.05	>0.20	>0.80
P_{50} (mm Hg)	Control	Mean	31.82	28.75	23.58	22.43	21.92
		SD	1.71	3.14	2.03	0.89	1.04
		SE	0.57	1.11	0.72	0.30	0.35
		n	9	8	8	9	9
	Resin	Mean	32.13	31.65	30.80	24.67	23.48
		SD	1.47	2.57	2.27	1.95	1.84
		SE	0.49	0.91	0.76	0.65	0.65
		n	9	8	9	9	8
		df	16	14	15	16	15
		t	0.41	2.02	6.89	3.12	2.17
		p	>0.50	>0.05	<0.002	<0.01	<0.05

			Day 0	Day 7	Day 14	Day 21	Day 28
Blood pH - 37 C	Control	Mean	7.032	6.899	6.822	6.741	6.715
		SD	0.036	0.034	0.087	0.038	0.036
		SE	0.012	0.011	0.029	0.013	0.012
		n	9	9	9	9	9
	Resin	Mean	7.071	7.020	6.945	6.882	6.867
		SD	0.037	0.028	0.041	0.043	0.035
		SE	0.012	0.010	0.014	0.014	0.012
		n	9	8	9	9	8
		df	16	15	16	16	15
		t	2.28	7.95	3.83	7.45	8.83
		p	>0.02	<0.002	<0.002	<0.002	<0.002
Blood Glucose (mg/dl)	Control	Mean	357	336	264	213	176
		SD	71	76	59	77	46
		SE	27	27	20	26	16
		n	7	8	9	9	8
	Resin	Mean	348	277	223	187	141
		SD	57	71	49	26	29
		SE	19	25	16	9	10
		n	9	9	9	9	8
		df	14	15	16	16	14
		t	0.30	1.71	1.63	0.94	1.84
		p	>0.50	>0.10	>0.10	>0.20	>0.05
Blood Lactate (uM/ml)	Control	Mean	3.63	8.46	14.30	21.20	28.90
		SD	1.68	2.48	2.48	6.72	15.60
		SE	0.56	0.83	0.83	2.24	5.90
		n	9	9	9	9	7
	Resin	Mean	3.33	9.14	15.60	23.40	34.80
		SD	1.23	1.55	2.23	6.90	14.70
		SE	0.41	0.52	0.79	2.30	5.20
		n	9	9	8	9	8
		df	16	16	15	16	13
		t	0.43	0.71	1.17	0.67	0.76
		p	>0.50	>0.20	>0.20	>0.50	>0.20
Blood Phosphate (mg/dl)	Control	Mean	10.80	15.89	20.38	23.94	21.18
		SD	2.31	6.14	3.62	2.80	5.44
		SE	0.77	2.05	1.21	0.93	1.92
		n	9	9	9	9	8
	Resin	Mean	15.06	17.88	21.81	29.19	27.46
		SD	1.96	3.64	5.67	4.79	4.55
		SE	0.65	1.21	1.89	1.60	1.61
		n	9	9	9	9	8
		df	16	16	16	16	14
		t	4.20	0.84	0.64	2.83	2.11
		p	<0.002	>0.20	> 0.80	<0.02	> 0.05

			Day 0	Day 7	Day 14	Day 21	Day 28
Plasma Phosphate (mg/dl)	Control	Mean	12.00	14.30	16.20	16.00	19.00
		SD	2.67	5.20	3.80	3.30	3.60
		SE	0.89	1.72	1.26	1.10	1.28
		n	9	9	9	9	8
	Resin	Mean	15.90	21.80	23.90	23.70	23.60
		SD	2.93	3.50	3.80	4.90	6.10
		SE	0.98	1.17	1.26	1.62	2.16
		n	9	9	9	9	8
		df	16	16	16	16	14
		t	2.92	3.59	4.32	3.91	1.83
		p	< 0.01	< 0.01	< 0.002	< 0.002	> 0.05
Blood pCO_2 (mm Hg, 37 C)	Control	Mean	76.5	92.3	110.5	95.7	72.5
		SD	6.6	10.0	22.1	13.9	7.0
		SE	2.2	3.3	7.4	4.6	2.3
		n	9	9	9	9	9
	Resin	Mean	70.2	64.1	72.6	80.0	66.6
		SD	10.3	12.9	15.3	16.6	6.9
		SE	3.4	4.6	5.1	5.5	2.4
		n	9	8	9	9	8
		df	16	15	16	16	15
		t	1.53	5.07	4.24	2.17	1.78
		p	> 0.10	< 0.002	< 0.002	> 0.02	> 0.05

TABLE 2

^{51}Cr Survival In Vivo of Baboon Red Cells Stored at 4 C for 21 to 28 Days With or Without Resin

Baboon	24-Hour Survival (%) Without Resin	24-Hour Survival (%) With Resin	T_{50} (Days) Without Resin	T_{50} (Days) With Resin
21 DAYS AT 4 C				
1	87	77	---	14.8
2	86	91	12.5	----
3	83	86	12.5	14.9
4	86	81	14.5	13.9
5	83	92	15.9	15.3
6	72	90	15.5	14.7
Mean	82.8	86.2	14.2	14.7
SD	5.6	6.0	1.6	0.5
SE	2.3	2.5	0.7	0.2
n	6	6	5	5
Paired t	1.20		0.12	
p	NS		NS	
28 DAYS AT 4 C				
7	71	--	12.1	----
8	78	--	17.8	----
9	77	--	----	----
Mean	75.3	--	15.0	----
SD	3.8	--	4.0	----
SE	2.2	--	2.9	----
n	3	--	2	----
10	--	83	----	14.1
11	--	77	----	14.7
8	--	85	----	11.6
Mean	--	81.7	----	13.5
SD	--	4.2	----	1.6
SE	--	2.4	----	1.0
n	--	3	----	3
Non-Paired t	1.97		0.50	
p	NS		NS	

FIGURE 1

^{51}Cr survival of baboon red blood cells stored with or without resin for 21 days at 4 C.

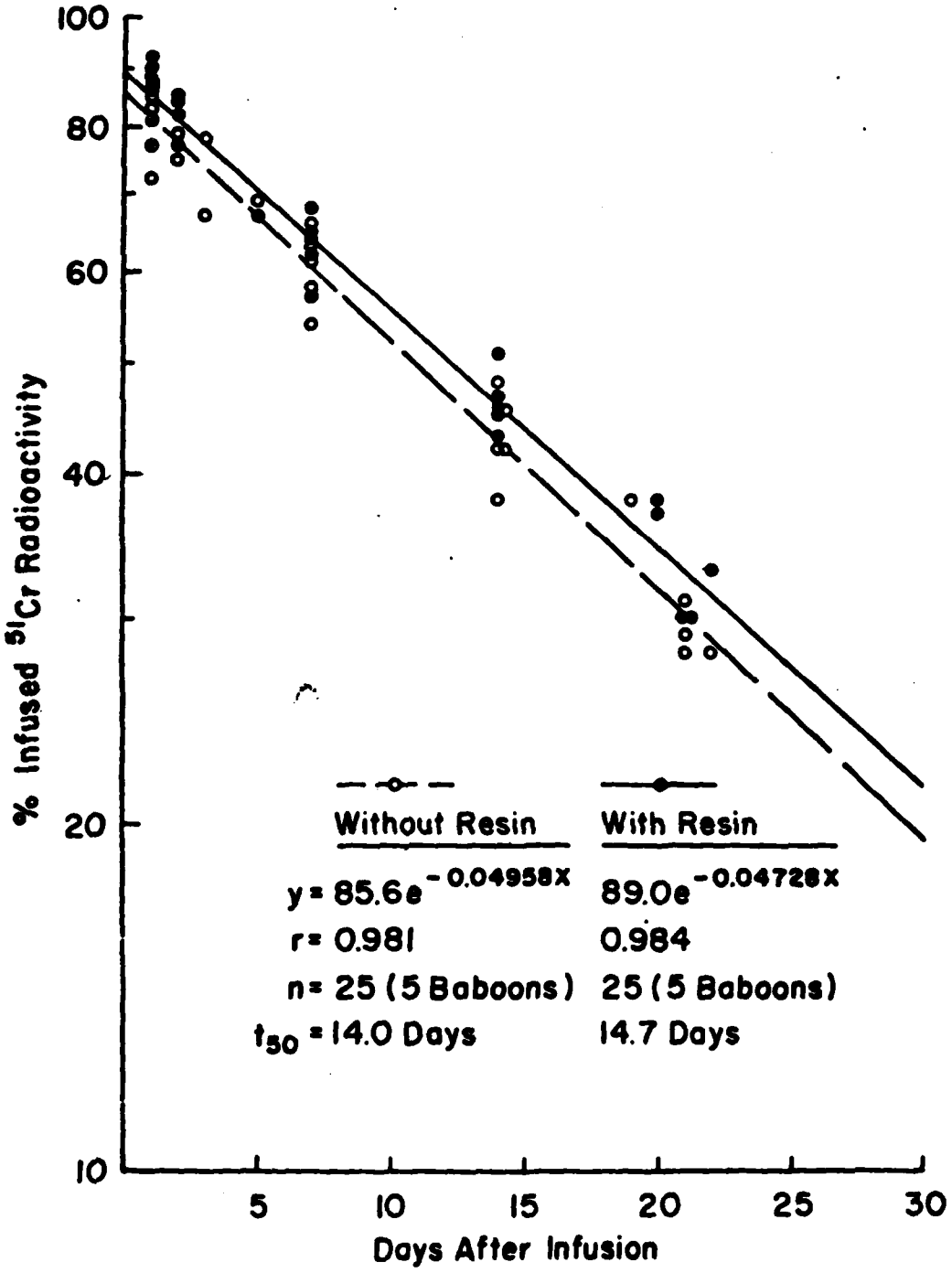

FIGURE 1

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FIGURE 2

^{51}Cr survival of baboon red blood cells stored with or without resin for 28 days at 4 C.

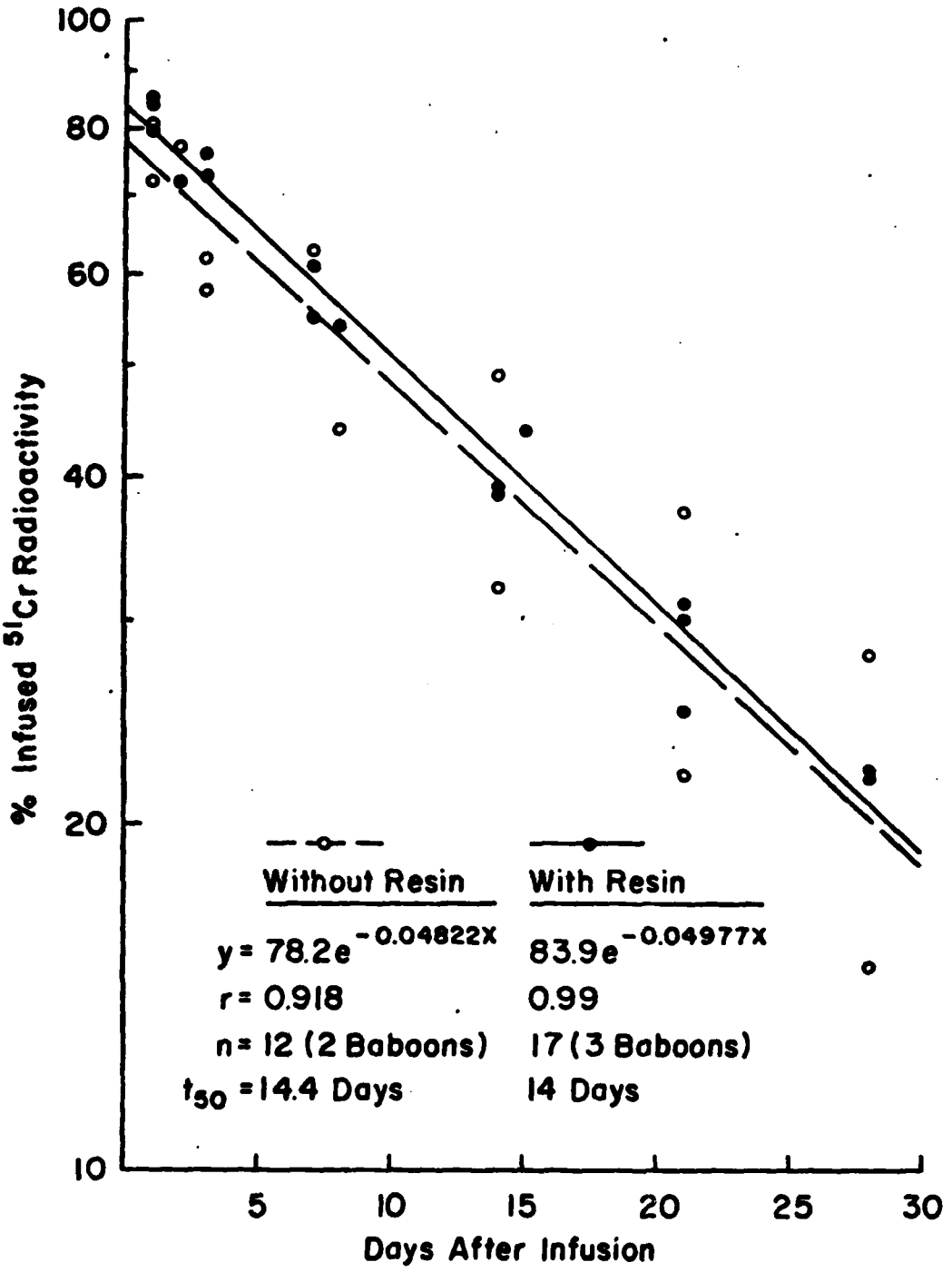

FIGURE 2

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